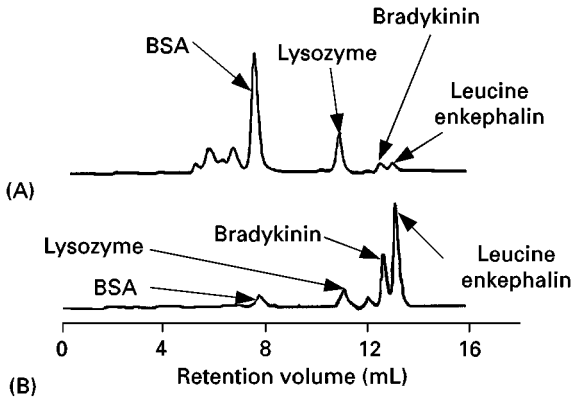

Figure 6 Chromatograms obtained simultaneously from the multiangle light scattering detector (A) and the refractive index detector (B).

Table 1 Peptide and protein mass values from the multiangle light scattering detector

Peak	*Solute*	M_r (*sequencing*)	M_r (*measured*)
1	BSA	67 000	64 300 ± 700
2	Lysozyme	14 300	14 600 ± 300
3	Bradykinin	1 060	1 090 ± 10
4	Leucine-enkephalin	556	592 ± 6

Table 1, which also includes relative molecular mass data obtained from sequencing the solutes.

It is seen that fairly accurate values for relative molecular mass can be obtained for the larger molecules, which can be extremely useful when dealing with completely unknown biopolymers. The errors involved are significantly greater for the materials of smaller relative molecular mass because the response (the amount of light scattered) is much less, and the output signal is much closer to the noise level of the sensing system. Further discussion of these types of detector are furnished in references provided in the Further Reading section.

See also: **II/Chromatography: Liquid:**Detectors: Evaporative Light Scattering. **Chromatography:** Protein Separation. **Appendix 1/Essential Guides for Isolation/Purification of Drug Metabolites. Essential Guides for Isolation/Purification of Enzymes and Proteins.**

Further Reading

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Hydrodynamic Chromatography

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Summary

Hydrodynamic chromatography (HDC) is one of the many techniques for particle size determination in the micron range. It has some similarities with size exclusion chromatography and field flow fractionation, but needs only one phase and one field. The main advantages are the separation of species according to size only, rapidity of measurement in the untreated medium and ease of operation of equipment. Variation in operating parameters allows a considerable range of possible applications. Disadvantages are low resolution, necessity for peak dispersion correction and calibration for signal intensity according to size. It has many applications in latex production and quality control.

Definition and General Features

Definitions

Particles may be separated according to their size by several techniques: HDC is one of them. This interesting rapid method (about 10 min) separates and sizes solutes or particulates in the micron range (0.030–60 μm) at a high dilution, without being

affected by their density. Particle size distributions (PSD) and their averages may be obtained. The separation takes place in packed (PHDC) or in open tubular capillary columns. Components are eluted in the order of decreasing size, as in size exclusion chromatography (SEC), a method which has many features in common with HDC. HDC appears as a complement, either to SEC, which is limited to small size and solute species, or to other fractionation processes based on the effect of an external field, e.g. field flow fractionation (FFF). They are all transport methods, but differ in order of elution and domain of separation. Resolution is examined below. Other techniques for size measurements are static ones and can be used to check the validity of the results from HDC. It is necessary to combine different measurement processes, according to the conditions and purposes required: to obtain a relative or absolute value, with or without separation, affecting or not the state of the sample, and depending on the size and resolution required.

In HDC the key value for a given particle of diameter D_p is called the separation factor R_F. This is the ratio of the highest elution volume (V_m) to that measured for this particle (V_p):

$$R_F = V_m/V_p \qquad [1]$$

This factor is greater than one, the inverse of the situation in thin-layer chromatography (TLC), where the solvent migration is more rapid than that of solute. By plotting the graph of known D_p versus R_F a calibration curve is obtained. This allows interpolation of the diameter of an unknown sample (**Figure 1**A). Another presentation is similar to that of SEC, log D_p versus V_p (Figure 1B) or D_p versus $(V_m - V_p)$. Other terms, detailed below, have the same meaning as in general liquid chromatography. Despite interest in this method, there are only a few papers (around 200) in the literature, as compared to separation by other chromatographic principles. Those that do exist are related to theoretical as well as practical aspects. General presentation may be found in papers by Barth, Hamielec and Tijssen. Currently, effective applications and use are increasing.

Origin

The first fundamental work was published by Small in 1974. The term hydrodynamic refers to the main driving force for separation. Under certain conditions, the nonturbulent flow in the column can be considered to be Poiseuille flow. Laminar flow occuring at a Reynolds number less than 2000 ($Re = 2R\bar{u}\rho/\mu$, where R = tube radius, $\bar{u}$ = velocity, μ = viscosity and ρ = density of medium), leads to a parabolic velocity profile in which the highest velocity is the centre of the tube (**Figure 2**A). For geometric reasons larger particles are statistically located preferentially in the axis of the capillary, whereas smaller ones are close to the walls. This difference in flow velocity is one of the separation mechanisms. Electrostatic effects must also be taken into account (Figure 2B).

The term separation by flow was initially proposed. Liquid exclusion chromatography has been used with porous packing. HDPC refers to a combined permeation and hydrodynamic process on small pore particles and porous hydrodynamic chromatography is used in packing with large pores. For capillary chromatography in more restricted conditions, another term has been used: tubular pinch chromatog-

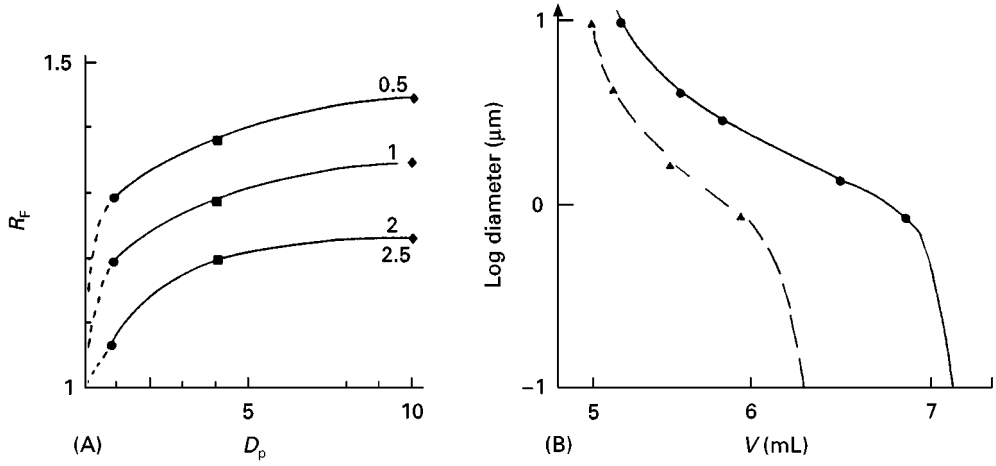

Figure 1 Calibration curves: (A) R_F versus sample diameter (D_p) at flow rates 0.5–2.5 mL min^{-1} and (B) log sample diameter versus elution volume (V). (Reproduced with permission from Revillon A and Boucher P (1989) Capillary hydrodynamic chromatography: Optimization study. *Journal of Applied Polymer Science*: *Applied Polymer Symposium* 43: 115.

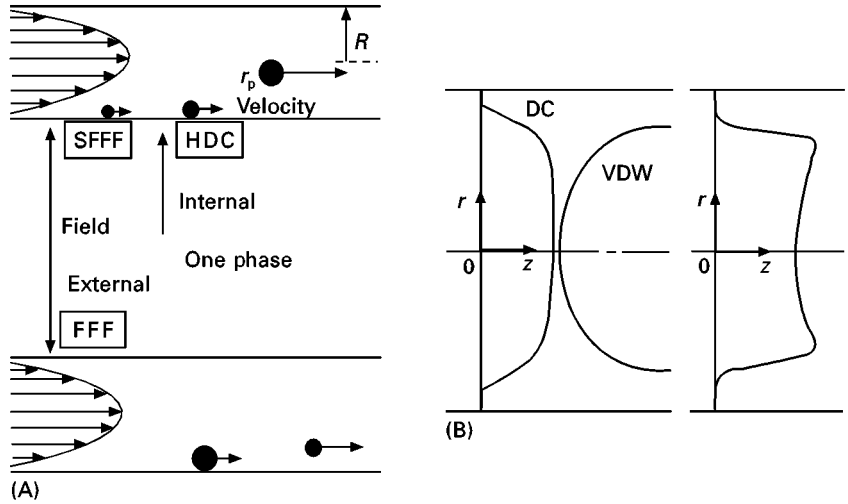

Figure 2 (A) Radial velocity in laminar Poiseuille flow (SFFF, steric FFF) and (B) concentration profile of particles under the effect of double-layer (DC) and van der Waals (VDW) forces (*r*, variable along radius of column; *z*, direction of flow). (Reproduced with permission from Revillon A (1996) Chromatographie hydrodynamique à colonne capillaire et à colonne remplie. In: Cavaille JY, Garcia-Ramirez M and Vigier G (eds) *Polymères: de la polymérisation aux propriétés*, pp. 167–174. Paris: Polytechnica.)

raphy. In slalom chromatography, the hydrodynamic effect in the interstices between particles leads to increasing elution volume with sample size.

A more frequently used alternative is hydrodynamic fractionation, since the process involved is not a classical chromatographic one of mass distribution equilibrium between two phases. In fact, there is only one phase, the mobile phase as an eluent, and only one field, the hydrodynamic one (Figure 2A). In FFF, a set of techniques for particle sizing, another external field is added. The intention is to modulate the intensity of the second field to inverse the elution order (e.g. in steric FFF). In HDC with packed columns, the nonporous stationary phase is theoretically inert. For porous packings, which allow the use of smaller capillaries, the SEC process operates simultaneously with HDC. Improvements on the original process are described in many papers and have been developed mainly at Lehigh University by Silebi and co-workers.

Advantages and Limitations

The main advantages of HDC are that it is a rapid and convenient method for the separation of particles. It allows one to obtain a fingerprint of the size distribution with an easy-to-operate instrument, similar to those used in liquid chromatography, at room temperature. Direct analysis of the original colloid medium and the use of high dilutions avoid modification of a sample which may be observed in a dry state (e.g. shrinking under the beam in transmission electron microscopy), and any effect of interactions. Existence of a unique, universal calibration curve allows calculation of D_p and PSD for any sample, since there is no effect of sample nature, surface charge and density on elution volume. At low ionic strength, no effect has been found of the chemical nature of the sample for vinyl copolymer colloids, even for those of low glass transition temperature, T_g. On the other hand, at high ionic strength, sample chemistry may present an additional parameter for separation. With this technique, there are none of the limitations encountered in liquid chromatography (solvent nature, stability and availability of stationary phase and temperature range).

However, there are some difficulties related to the proper choice of operating variables. Elution has been shown to depend on size and porosity of packing beads, eluent flow rate, ionic strength, pH and additives such as surfactants. It is evident that column length and diameter play a role on plate numbers, resolution and domain of measurement. Particle size may affect the total recovery of material (with packed columns). Moreover, it may act on the detector response. In consequence, the PSD might be affected by the incomplete recovery of particles, due to adsorption effects, mainly for larger particles. For example, total recovery is observed up to 200 nm only with 20 μm packing.

Intrinsic limitations include a low plate number, N, and low resolution, R_S, so that generally the number of peaks (peak capacity p) in a chromatogram is low (about 5–10). Quantitative interpretation for determination of particle size distribution needs calibration in order to establish correspondence between

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sample size and elution volume and the relationship between signal intensity and amount of particles. Moreover, band broadening, common to every chromatographic process, has a larger influence on PSD because of the low resolution. The interpretation of data assumes that particles are spherical, although an equivalence has been found for elongated structures (1 μm particles appear as spheres of 0.153 μm diameter). Finally, soft materials may be deformed under the high rate of shear in packed column and orientation in flow may affect the apparent size.

Applications

There is no limitation to the nature of the sample, but most studies are in the polymer field. In control and in research, particle characterization is a necessity and numerous chemical and physical data have to be determined, such as molecular weight distribution (MWD) and averages ($\bar{M}$) or PSD. These values are key parameters for determining rheological, mechanical, thermal and optical properties, storage stability, film-forming capability and the general behaviour of polymer materials. MWD and $\bar{M}$ are commonly measured by SEC and particle size may be obtained by a variety of methods.

To reduce use of organic solvents in the production of polymers (by bulk and in organic solvent processes), free radical suspension and emulsion polymerization heterogeneous processes are used. The first process leads to larger bead sizes (around mm), which are not dealt with in HDC but HDC is typically suited for latex evaluation (around 100 nm) obtained by the second process. These polymer particles are used either largely in water-borne paints, inks and relatively low cost coatings, or as high value colloids for model compounds. For instance, they are used as standards for calibration (membranes) or in biochemistry for diagnostic aids and purification, or for packing chromatography columns. Much of the practical HDC work has been devoted to synthetic organic colloid separation and diameter measurements for quality control. Separation of natural products such as proteins is also of interest. The rapidity of the measurement is compatible with kinetic studies and monitoring during the polymerization process. Swelling of carboxylic latexes has been measured according to pH. The stability of mini-emulsions (50–500 nm) has been studied successfully. Flocculation of colloids in the presence of water-soluble ionic polymers or inorganic oxides has been observed by HDC in conjunction with other methods. Association of particulates under the effect of a thickener is clearly demonstrated, though it can be broken by intensive shear, and the same applies to aggregates.

Some authors have attempted to determine molecular weight or size for very large polymers, for instance water-soluble ones, like polyacrylamide, xanthane, polysaccharides and tobacco mosaic virus. The flow and dynamic behaviour of macromolecules in packed bed have been studied.

A variety of other compounds have been examined, such as carbon black, paper fibres, cement, clay, metals and oxides of Fe, Ti, Si, Al, silver halides and biomaterials such as milk or liposomes from egg yolk lecithin. Silica has been extensively used since it has the advantage of being a hard spherical model for HDC mechanism studies.

Moreover, HDC is of interest in the fundamental study of flow behaviour in tubing or pores which are encountered in transport technology of materials. Applications are also found in geology.

Equipment

Instrument

The instrument is similar to a liquid chromatograph: solvent source, pump, injection valve and column. Optical detection is spectrometry, refractometry, turbidimetry or light scattering.

Conditions to be satisfied are accurate flow rate control and high detection sensitivity. The first condition is due to the narrow elution domain and the second to the small injected volume of a very dilute sample.

Optical detection is more difficult since particle dimensions are in the range of the wavelength involved, λ. With a UV spectrometer, sensitivity is so high that as few as 25 particles in the detector cell give a noticeable signal. Sensitivity varies with wavelength, sample type and with sample size since the signal depends on absorption and scattering, which vary with sample diameter to the power a. The result is that quantitative interpretation needs preliminary signal calibration for each species.

A general formula in liquid chromatography for the instantaneous detector response H at each elution volume V is:

$$H = \sum N_i(V) D_i^a(V) K_i(V) \qquad [2]$$

where K_i is the extinction coefficient for particles of diameter D_i and $a = 2$ in the Mie scattering regime for a turbidity detector. In the Rayleigh regime, valid for $\lambda/D_p < 0.3$, $a = 6$ for turbidity and 3 for refractometry. Hamielec observed a factor 6 in turbidimetry and a linear increase of signal with particle size in refractometry: there was a three times increase between 100 and 500 nm for polystyrene (PS) collo-

ids. For the same given weight of PS colloids, the UV signal is three times higher for 354 nm than for 88 nm particles. This also explains the effect of wavelength on signal shape and intensity. The peak separation is better with a refractometer, which may appear less sensitive than the turbidity detector. Generally, $\lambda = 254$ nm is chosen, but differences in relative sensitivity at $\lambda = 380$, 254 and 220 nm for PS colloids of 38 nm and 176 nm diameter have been found; this variation for larger PS colloids, 0.84 μm and 4 μm diameter, was also observed. A continuous and moderate increase in specific extinction coefficient at 254 nm and a higher and nonmonotonous increase at 220 nm have been measured. A low signal obtained for small particles may be enhanced by working at lower wavelength so that the apparent separation is increased. Difference in absorption may allow the apparent separation of PS/PVC. Intermediate values of a may correspond to a combination of a and $K(V)$. The value $a = 1$ holds for refractometry and spectrophotometry of polymers in SEC, so that the range 1–6 must be considered for a general data treatment.

Assuming the formulas for number and weight average diameters:

$$\overline{D_n} = \sum ND / \sum N \quad [3]$$

$$\overline{D_w} = \sum ND^4 / \sum ND^3 \quad [4]$$

and the simplified general relationship H proportional to ND^n, (K and a are independent of V), then:

$$\overline{D_n} = \sum (H/D^{n-1}) / \sum (H/D^n) \quad [5]$$

and:

$$\overline{D_w} = \sum (H/D^{n-4}) / \sum (H/D^{n-3}) \quad [6]$$

For all the investigated PS latexes, average diameters of the number and weight greatly decrease when n is increased (**Figure 3**). The best agreement with values measured with transmission electron microscopy is obtained for $n = 3$–4, which is in agreement with theory since their diameters are in the range of the wavelength of detection (254 nm). Except for $n = 2$, the polydispersity index $P = \overline{D_w}/\overline{D_n}$ is not affected by n, and is the same for all samples. Nevertheless, these distributions do not correspond to true ones. The high polydispersity values compared to those determined by transmission electron microscopy (1.02) clearly show the necessity of a band-broadening correction.

A number of workers have built apparatus for HDC by putting together high performance liquid chromatography components. Matec Applied Scientific (Hopkington, MA, USA) currently markets a high resolution instrument CHDF-1100 with UV detection. Separation is operated in a narrow capillary (2.5 μm × 10 m) enclosed in a cartridge. Careful choice of eluent components avoids blocking the column for long-term use. Control of temperature allows accurate flow rate. The signal resulting from absorption and scattering is treated with the help of Mie theory. A mixture of small size latexes is well separated in about 10 min. Length of column may be increased, and diameter may be changed to cover different particle sizes. The apparatus may be used in process control.

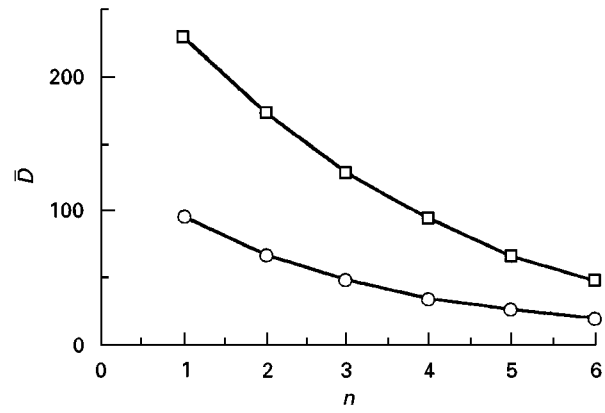

Figure 3 Effect of n value on average diameters: number (circles) and weight (squares) for standard PS latex 106 nm. (Reproduced with permission from Revillon A and Guilland JF (1990) Software for data acquisition and treatment: hydrodynamic chromatography (HDC) analysis. *Journal of Applied Polymer Science: Applied Polymer Symposium* 45: 125–137.

Improvements to enhance the accuracy of R_F and PSD, resolution, sensitivity of detection and rapidity of analysis include the following:

1. The injection valve may be replaced by a 12-port valve and a delay column, with two injection loops (Valco Europe, Vici, Switzerland). This allows the injection of two (identical or different) samples on one or two consecutive columns. One of the advantages is the accurate determination of R_F by the help of an internal standard, avoiding sample mixing or reaction, and peak interference. Moreover, the comparison of the signals of the sample passing through active and inert columns allows determination of percentage particle recovery.
2. An additional fine metering valve (Vernier Handle, 18/21Y, Hoke, Creskill, NJ, USA) may be placed before the packed column to divide the main flow into two parts, one entering directly

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into the column and the other through the injection valve. This reduces peak asymmetry from 5 to 1.5 and increases plate number by a factor of two. The optimum effect is obtained when the flow is divided in two equal parts; the main flow is less diluted and the side flow entering the column, close to the injection point, prevents broadening of the liquid jet containing the sample.

3. Effort has been put into the reduction of void volume, by direct column injection, design of a detector with reduced connection and cell volumes, on-column detection, improved sensitivity and the use of micro-equipment similar to that used in liquid chromatography and SEC.
4. Recycling, as used in SEC, may improve resolution or R_F determination without changing or increasing the R_F value.
5. A diode array UV-visible spectrometer allows simultaneous determination of the signal at several wavelengths, which means either signal enhancement or decrease (masking of a component).
6. Laser light scattering is of high sensitivity and may also be used, combining photon correlation spectroscopy with integration of the signal, at one angle, during a low counting time (a few seconds). It may give directly both the instantaneous size and concentration of the sample. Low angle laser light scattering is the detector used to determine the molecular weight of polyacrylamide. Variants of this include the evaporative light-scattering detection and condensation nucleation light-scattering detection. To overcome a calibration problem and use of standards, online viscosity has been proposed as an alternative, but this method awaits the development of improved pressure transducers. Fluorometry has been suggested as a high sensitivity detector for tagged polymers. The approach in the author's laboratory is discontinuous measurements of sample size on eluted fractions by transmission electron microscopy, photo correlation spectroscopy and sedimentometry.

Dispersion Correction

Even with micro-equipment and enhanced resolution, selectivity generally appears low. Results must be corrected for band spreading, in order to get individual peaks and true distribution.

There are many approaches for solving the dispersion problem based on Tung's equation, which expresses the experimental chromatogram, $F(v)$, as the result of the true chromatogram, $W(y)$, times a dispersion function, $G(v-y)$:

$$F(v) = W(y) * G(v-y) \qquad [7]$$

This equation has received integral and numerical solutions. It has been shown that skewed instrumental spreading functions derived from the plug flow dispersion model fit data for particle separations by HDC, where the spreading function is:

$$G(v, y) = \{4(\pi \mathrm{Pe}^{-1}(v/y))\}^{-0.5} \times \exp\{-(v-y)^2/4\mathrm{Pe}^{-1}(v/y)\} \qquad [8]$$

(where the dispersion term or Peclet number, $\mathrm{Pe} = UL/D'$) when U = superficial velocity, L = length of the packed bed, D' = empiric dispersion coefficient; v = elution volume and at the maximum of peak = y). Increasing Pe leads to narrower peaks.

Pe = 100 corresponds to no peak correction. With packed column, whatever the value of n, $\overline{D_n}$ increases with Pe, the effect is stronger for higher exponent, n. On the other hand, $\overline{D_w}$ increases or decreases with Pe, depending on n. A quasi-constant value corresponds to $n = 3$–4 (**Figure 4**).

In another representation of results for different Pe values, $\overline{D_w}$ versus n curves for all samples have a common intercept between $n = 3$ and 4. A rapid change of D is noted up to Pe = 500, then $\overline{D_n}$ and $\overline{D_w}$ tend to converge slowly, independently of n.

Practically, considering the whole set of results with UV detection, n must be chosen as 3–4 and Pe 500, to get the best average and distribution values, as well as narrow peaks, which means enhanced resolution.

Experimental Parameters

Sample is put in the liquid used as the eluent; the eluent which must be filtered (e.g. 0.22 μm: Millipore) to prevent contamination and ensure long-term reproducibility. No other specific treatment is needed. Weight concentration is in the range of 10^{-4}, depending on the detector. This concentration does not need to be known exactly. A small amount of this solution or suspension is transferred with a syringe into the loop of a six-port valve. Loop capacity is typically from 1 to 50 μL. Samples are injected separately or in mixtures. It is necessary to ensure high accuracy in data acquisition, since the useful elution domain is narrow. Frequent flow rate calibration is also necessary, with the ability to correct small changes in elution volume. With most detectors, the signal increases linearly with concentration or injected volume, in a range useful for application. This means, that no interaction, adsorption or overloading occurs. Peak characteristics are not affected.

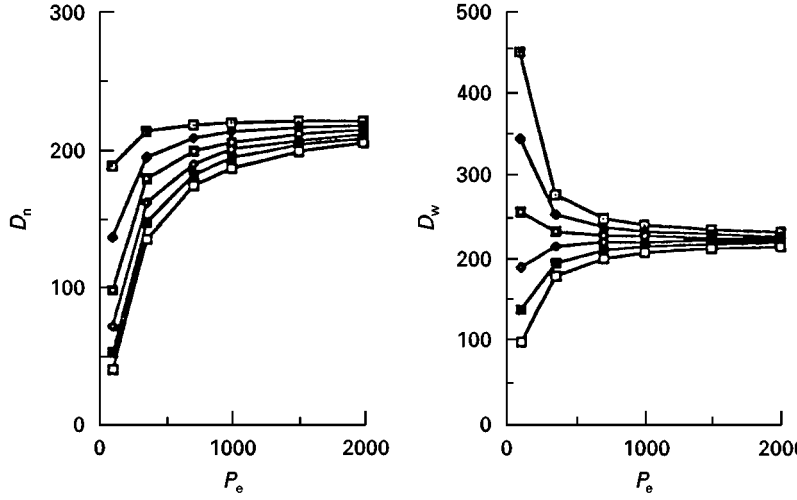

Figure 4 Effect of n and P_e values on average diameters: number and weight for standard PS latex 234 nm. (Reproduced with permission form Revillon A and Guilland JF (1990) Software for data acquisition and treatment: hydrodynamic chromatography (HDC) analysis. *Journal of Applied Polymer Science: Applied Polymer Symposium* 45: 125–137.

For packed columns, the usual eluent is deionized water with additives, such as traces of formaldehyde to prevent bacterial contamination. A variety of ionic (e.g. sodium lauryl sulfate, about 1 g L^{-1}) and nonionic (AOT/Rohm & Haas, Brij, Triton/American Cyanamid) surfactants may be used at a concentration of 1–10 mmol L^{-1}. Ionic strength is preferentially ensured by sodium nitrate (0.5 mmol L^{-1}). A buffer may be added. Organic solvents may have interesting thermodynamic features and have been used for some applications.

Column dimensions are 0.8–1.1 m in length and even 15–50 cm, with inner diameter from 2 to 10 mm. Sometimes two shorter columns, which are easier to pack, are assembled in series. Glass may be the column material, but stainless steel is more common. Column packing diameter has decreased from 20–100 to 5–10 μm, so that the number of plates is about 30 000 for a 0.5 m column. $N = 42\,000$ is obtained with small particles of 1.4 μm and 15 cm length. Flow rate varies from 0.07 to 0.7 mL min^{-1}, but is generally about 1.5 mL min^{-1}.

With capillary columns, aqueous and organic eluents have been tested with various additives in the aqueous eluents similar to those described above. Organic eluents may be advantageous because of their physical properties (viscosity, refractive index, solvating power) but the preferred eluent is water. Flow rate varies from 0.1 to 3 and even 9 mL min^{-1}, but is generally about 1 mL min^{-1}.

The reference sample, used as marker for determination of maximum elution volume V_m, is generally $Cr_2O_7K_2$ or $Cr_2O_7Na_2$, giving a high intensity signal. Compounds such as salicylic acid with the fluorometer or tritiated water may also be used.

Reference materials for calibration are monodisperse PS latexes, prepared by emulsion polymerization in the laboratory or commercially available. Quantitative measurements on peaks are: height (H), base width (w), half-height width ($w_{1/2}$), skewing or asymmetry factor ($sk = a/b$, ratio of second to first part of the peak width at $h/10$), absolute (V_e) and relative (R_F) elution volumes.

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Liquid Carrier

The effect of additives in water (surfactant, ionic strength and viscosifying agents) will be considered here.

Surfactant Colloids must be stabilized sterically and/or electrostatically. The surfactants are either bonded or adsorbed on the particles; in the latter case, a part passes in the dispersing phase when dilution takes place (for injection and during the elution) so that flocculation can occur. Moreover, experiments with packed columns have shown that surfactants play a role in elution. Silebi observed a decrease of R_F when concentration is increased, but no change in the slope of log D versus ΔV. This is shown in **Figure 5**, for R_F at various amounts of sodium dodecyl sulfate (mmol L^{-1}) in the presence of 0.2 mmol L^{-1} NaCl.

Sodium dodecyl sulfate is frequently used below or above its critical micellar concentration (2.5 g L^{-1}). This agent provides wettability of beads and ensures a low ionic strength (0.01). This favours higher R_F, better elution and increased resolution. The effect is greater for larger particle sizes. Other work has found that nonionic surfactants lead to higher R_F than

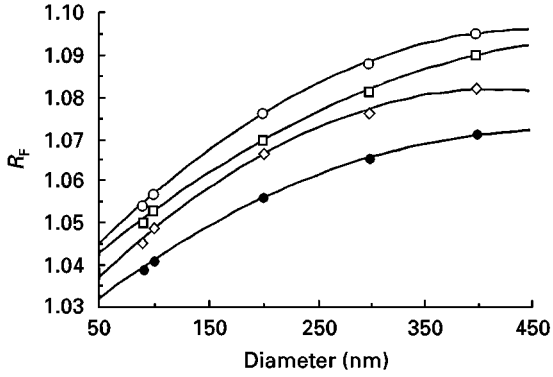

Figure 5 Effect of surfactant (SDS) on R_F, in the presence of NaCl 0.2 mmol L^{-1}. Open circles, 0.13 mmol L^{-1}; open squares, 0.26 mmol L^{-1}; open triangles, 0.52 mmol L^{-1}; filled circles, 1.08 mmol L^{-1}. (Adapted with permission from results of Silebi CA and MacHugh J (1978) In: Becher P and Yudenfreund MN (eds) *Emulsions, Lattices and Dispersions,* pp. 155–173. New York: Dekker.

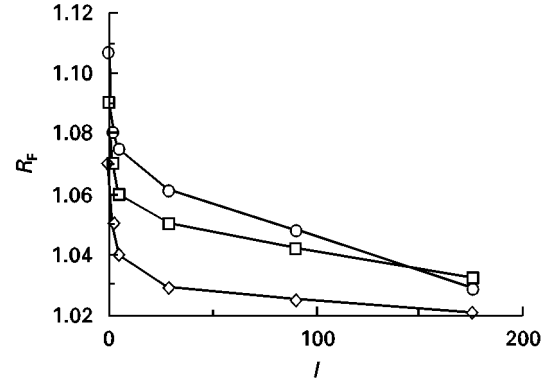

Figure 6 Effect of ionic strength I (Na_2HPO_4) on R_F for three PS latexes of diameter 100 nm (triangles), 200 nm (squares) and 300 nm (circles) in the presence of 2.67 mmol L^{-1} surfactant. (Adapted with permission from results of Silebi CA and Mac Hugh J (1978) In: Becher P and Yudenfreund MN (eds) *Emulsions, Lattices and Dispersions*, pp. 155–173. New York: Dekker.

ionic ones, whereas stabilization by anionic surfactants is claimed to be considerably more effective than with nonionic ones. It is important to bear in mind that molecules of surfactant are in equilibrium with micelles above the critical micellar concentration. Depending on the amount and nature of surfactant in the sample, a new equilibrium between sample and eluent may explain the differences in observed results. It has been reported that SDS, tested from 0 to 4.8 g L^{-1} improves baseline, peak shape and reproducibility and increases R_F in capillary columns.

Ionic strength Electrolytes may have four effects:

1. on the limiting R_F value
2. on percentage recovery
3. on the size domain of interest
4. on sample chemical composition

Electrolytes greatly modify the critical micellar concentration of the surfactant and affect the repulsive electrostatic double layer around the particles. Basically, a low ionic strength I is necessary for screening charges. Recall that:

$$I = 0.5\sum c_i Z_i^2 \qquad [9]$$

where c_i = total concentration of species i, of valency Z_i.

1. Increasing the ionic strength, I, leads to a decrease in R_F (**Figure 6**). In the initial work, even the ionic marker was affected: a 3% change in V_m was observed when increasing NaCl concentrations by a factor of 1000. Initial results obtained with packed columns have been confirmed by several authors, who observed a change in R_F with ionic strength I, due to competition mainly between van der Waals (attractive) and double-layer (repulsive) forces. At high ionic strength, elution is opposite to the normal mode. The reason could be that very strong van der Waals interactions dominate over the hydrodynamic effect. Some authors have observed a normal decrease in R_F when I increased, but no change in the slope of log D_p versus ΔV.
2. Percentage recovery is decreased for various colloids when I is increased on a 20 μm packing but is more constant for PS on large pore, large packings (63–125 μm Fractosil). A short column favours the recovery of large particles.
3. Change of I may make possible the separation or the elution of large particles (limit 850 nm instead of 500 nm), even if N or R_F is decreased.
4. In addition, the chemical composition of the sample can affect the elution, so that a mixture of polystyrene and poly(methylmethacrylate) (PMMA) latexes of identical size (240 nm) can be separated in the presence of 0.4 mol L^{-1} NaCl, on a 1 m column packed with 20 μm PS beads. On the other hand, there is no elution difference between latexes of PS and PMMA of diameters from 58 to 207 nm, when NaCl is varied from 4.44 to 15.6 mmol L^{-1}.

In capillary columns, no effect was observed for either $NaNO_3$ (0–28.6 g L^{-1}) or NaCl (0–48.7 g L^{-1}). Peak shape and elution volume of samples and marker were not modified – or only slightly – by variation in the salt concentration, but $NaNO_3$ has some absorption and affects the baseline.

Viscosity additives Some authors have observed a change in R_F mainly for larger particles, in the presence of ethylene glycol or sucrose. A small amount of ethylene glycol prevents aggregation. Theoretically, transverse motion must be decreased with decreased tailing and increase in R_F, when the viscosity is increased. In this respect, polymers have been used with some success. Neither increase in R_F nor shape improvement in peaks was observed in the range 0–6% ethylene glycol with capillary columns. The pressure is markedly increased, according to Darcy's law and a permeability coefficient, K_o, may be derived. For $L = 60$ m, a K_o value of 2.4×10^{-8} m^2 is far lower than that of filtration membranes (10^{-12} m^2), which indicates the low separating power of a capillary column.

As a result of these observations, only the surfactant SDS at 3 g L^{-1} was used as an additive in capillary columns.

Flow rate, Q and nature of the eluent Flow rate may have two effects: one on N and one on the sample.

A high flow rate may induce polymer deformation or degradation. It was found that under 2.5 cm s^{-1} no deformation of soft polymer is expected from a calculation using a Deborah number.

Increase of resolution by a factor of 2 has been observed with a 10 times decrease of flow rate, but the effect is small if the packing is of very small particle size. It is known that, in general, low flow rates favour minimum equivalent plate height, H_e, according to the van Deemter equation:

$$H_e = L/N = a + b/v + cv \qquad [10]$$

where v is linear velocity. Here, this general expression holds with zero as c value, since it represents the mass transfer term. In capillary HDC diffusion has been found to be $b = 7$ cm^4 min^{-1} and $a = 10$ cm for longitudinal and eddy terms, respectively. These values, which are far higher than in liquid chromatography, mean a high sinuosity coefficient and large equivalent bead sizes.

In capillary columns, N, R_S and R_F vary differently with eluent (water) flow rate Q, depending on sample size. Number of plates was found to increase with flow rate, but R_F decreases for a 4 μm sample and the resolution is slightly affected. Some deformation of peaks was observed at low flow rates. For instance, the resolution between 0.84 μm particles and marker or 4 μm particles was unchanged, but was decreased between 4 μm and marker. The interpretation may be found in the tubular pinch effect, by taking account of the respective Reynolds numbers of the particles, Re_p (see above). Their values correspond to very different flow rates so that a change in mechanism occurs in the investigated region.

These different results for R_S, N and R_F show that operating conditions are not rigid and they must be chosen as a function of the particular analysis. Depending on the objective, it may be required to obtain either rapid results in 1.5 min with medium resolution or higher resolution in 36 min. It is also interesting to note that the highest number of plates is obtained not only for the marker, but for the largest sample.

In methanol, the number of plates increased from a constant value for 0.84 and 4 μm particles and marker to a maximum for the 10 μm sample, when Q increased. The resolution, like R_F, decreased when flow rate, Q, was increased.

Tetrahydrofuran has low viscosity and is a good solvent for polymers. These characteristics may allow higher flow rates and the study of polymers in solution. In fact, although excellent baselines, very high number of plates and good chromatograms of crosslinked PS (10 μm) were obtained, the upper limit of R_F was decreased with this solvent. In tetrahydrofuran polybutadiene, polyisoprene and PS of nearly the same molecular weight (300 000) were eluted according to their respective dimensions, $r_g = 28.9$, 26.6 and 25 nm on a packed column.

Water, being a more versatile eluent, and offering a good compromise between R_F and N values, is the main solvent at a flow rate of about 1 mL min^{-1} with a capillary column. This flow rate also corresponds to a compromise between higher R_F (Figure 1A) and lower resolution at the higher velocities. **Table 1** summarizes the experimental conditions and results.

As an ancillary practical application, Poiseuille's law allows the determination of one of the parameters: flow rate Q, pressure P, length L, viscosity μ and capillary radius R:

$$\Delta P = 8\mu LQ/\pi R^4 \qquad [11]$$

Table 1 Experimental conditions with capillary columns

Length (m)	30	60	120	120	60	120
Radius (μm)	125	125	125	125	250	125
Eluent	W	W	W	Meth	W	THF
Maximum R_F	1.4	1.45	1.45	1.45	1.6	1.26
N (m)	500	900	1900	7000	400[a]	5300
Maximum N (m)	16.7	15	15.8	58.3	38.3	250

W, water: flow rate 1 mL min^{-1}, except [a]5 mL min^{-1}; Meth, Methanol; THF, tetrahydrofuran. Reproduced with permission from Revillon A and Boucher P (1989) *Journal of Applied Polymer Science: Applied Polymer Symposium* 43: 115–128.

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Columns

Capillary columns for capillary HDC are used for larger particles, generally in the range from 1 to 60 μm. New capillary columns allow separation in the sub-micron range. Packed columns are effective for smaller particles, with diameters of 30–1000 nm. Since its introduction, HDC studies have been mainly devoted to separations with packed columns.

Packed columns Columns may be slurry- or dry-packed in the same way as for liquid or gas chromatography. Packings used include ion exchange resins, cross-linked poly(styrene-divinylbenzene), nonporous glass and silica gel among others. As an example, analysis has been performed with a slurry-packed column (cross-linked PS spheres of uniform diameter 20 μm: 50 cm length, 0.78 cm internal diameter).

The main determining parameter is packing particle size. A general trend in liquid chromatography is an increase in plate number when particle diameter decreases (N is inversely proportional to the square of particle diameter). It has been shown in the initial work on HDC that resolution is increased by using packings of small monodisperse spheres; **Figure 7** shows this result. Moreover, resolution is also decreased when size increases (more than a factor of 2 between 20 and 40 μm). Whatever the size of the packing material, the elution region is very limited, defined by a maximum ratio R_F of 1.15 in most papers. By using 2 μm nonporous silica gel packing and tetrahydrofuran as eluent, a value of 1.21 is obtained for R_F, yet the chromatogram contains only five peaks: four for PS and one for toluene. R_F values as high as 1.3 have been obtained.

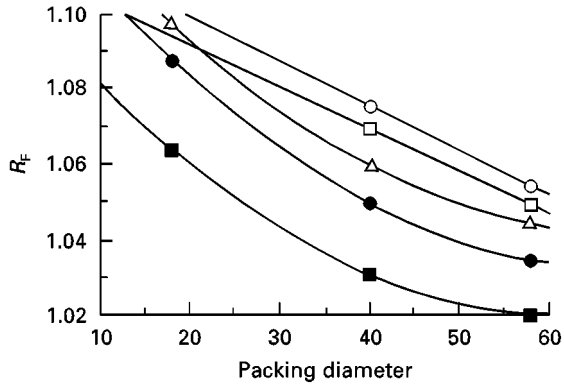

Figure 7 R_F versus packing diameter for five latexes. Open circles, 1000 nm; open squares, 800 nm; open triangles, 600 nm; filled circles, 400 nm; filled squares, 200 nm. (Adapted with permission from results of Small H (1974) *Journal of Colloid and Interface Science* 48: 147–161.

Large (125–180 μm) but porous particles are still used (at low flow rate: 2 cm min^{-1}, in the presence of 0.01 mmol L^{-1} NaCl) to take advantage of the pores as capillaries, with an R_F value of 1.16 or up to 1.39 in the measurement range 240–1230 nm. The equivalent capillary radius R is 2.8 μm, calculated according to the formula:

$$R = \frac{r_p}{1 - \sqrt{2 - R_F}} \qquad [12]$$

By combining HDC and SEC, and using porous particles, the R_F may be increased by a factor 1.1–1.2 to a value of 2 or 2.2. The peak broadening decreases or increases, so that resolution is inferior to that obtained on nonporous packing. Moreover, small size porous packings in SEC (2 μm Hypersil or polymeric 3 μm packing with 5 or 15 nm pore radius) allows a high plate number in a 0.45 cm column to be obtained, with an expected peak number of 66. Considering a resolution of unity, constant with V, the peak capacity is:

$$p = 1 + (R_F - 1)N^{0.5}/4R_F \qquad [13]$$

This leads to $p = 37$ in this case, and the number obtained is approximately 15, which is an excellent value. The authors of this work also used nonporous monodisperse particles, leading to the theoretical plate height minimum value (5 μm), with no dependence of flow rate in the range 3–7.5 cm min^{-1}. N is 42 000 for a 15 cm length column, with 1.4–2.7 μm silica particles so that a mixture of styrene polymers in the range of molecular weight 10^4–10^7 are well separated in 6 min, but shear degradation occurs for the higher molecular weight polymers.

Capillary columns With open capillary tubular columns, the resolution is poorer than with packed columns, but the R_F may be as high as 1.45, so that the peak capacity may be the same as that obtained with a packed column.

The main parameters are length and diameter. A systematic study of the length has been made with stainless-steel columns of 30, 60 and 120 m, and internal diameters of 0.25 and 0.5 mm. The 30 m column gave results of insufficient quality, so most of the work was done with the 60 m columns and finally optimized with 120 m length columns. By going from 60 to 120 m, the theoretical plate height was found to be unchanged and N increased from 380 to 800, for a 4 μm sample. A way to illustrate this increase in resolution is to consider the calibration as represented in SEC. A lesser slope (Figure 1B) allows a better separation.

Table 2 Characteristics of capillary hydrodynamic chromatography systems

Length (m)	*Internal diameter (mm)*	*N (m)*	*Maximum R_F max*
88–201	250–500	?	1.3
50	180–450	25	1.5
60–200	250–500	10	1.55
15	100	25–200	> 1.2
0.45–7	1000	6	1.5
0.15–0.2	1	250	1.1
0.7–3.3	1.2–10	105	1.05
91–168	250–500	16–100	1.4
12	15 000	?	1.15
30–120	250–500	16–250	1.5
2	4	2000	> 1.15
2	6.5	?	1.42
5	7	600	1.63
2.5	10	580	> 1.46

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The second characteristic of the column is its diameter. **Table 2** summarizes conditions of separation and typical results (N, R_F). It can be seen that capillary diameter varies considerably, from 1 to 1000 (or even 15 000) μm. Most of the initial work has been done with 250–500 μm internal diameter column. The choice of tube diameter corresponds to the different ranges of sizes to be separated. The limit in R_F shows that over a certain sample size, no separation occurs. This limit of size may be related to the ratio of the average radius of the sample, r_p, to the tube radius, R. A third order law, relating r_p to R, may have a reasonable approximation in a linear one in agreement with Small's observations:

$$r_p = f + kR \ (\mu m) \qquad [14]$$

with $f = -7$ and $k = 0.1$ (R and r_p in μm). A column of diameter 500 μm may have a medium range of separation of 18 μm instead of 5 μm for the 250 μm one. Taking into account the published results, the ratio of R to r_p is about 100, and for a given diameter of column, the usable r_p/R range varies roughly from 10^{-3} to 10^{-1}. The interest in large diameter columns is because of the decrease of rate of shear, γ, but the resolution is higher for narrow tubes.

To reduce extra-column band broadening, optimization of the injection-detection system has been attained with 50–100 μm capillaries judging by results obtained in capillary electrophoresis. With microcolumns, efficient separation has been obtained for PS samples with molecular weight of 10^3–10^6 Da. The chromatogram was similar to that of SEC, but with a limited R_F of 1.1 (instead of 2 in SEC) and a low number of plates ($N = 50$). Work by Tijssen shows a very high number of plates ($N = 10^5\ m^{-1}$), but a more limited R_F, of 1.05. The increase in N was not accompanied by an increase in R_F, so that the peak capacity remained low (less than 10). More recent work indicates higher values of R_F: 1.63 and rather good resolution between latex samples. Even with 4000 plates, the peak capacity is only about 7. **Table 3** summarizes the conditions and results observed with capillary and packed column HDC.

Mechanism and Other Methods

Some of the difficulties for M or PSD determination may be solved using other types of chromatography: liquid, FFF – normal and steric (SFFF) – supercritical fluid, capillary electrophoresis, or combining with other methods (thermal FFF). Countercurrent chromatography or centrifugal partition chromatography is a potential tool for separating copolymers of different structure or nature, but currently there are few examples. These methods correspond to a large variety of separation mechanisms based on kinetics or thermodynamics, where surface, volume, active specific sites of materials and thermal, gravity, electric, magnetic applied fields bring their contribution. They are also called fractionation and have features in common with chromatography. One characteristic is the absence of the stationary phase, which avoids the problems of interfering phenomena such as adsorption, mass transport, column channelling, degradation and shear rate. This allows a greater choice of eluent.

Some other solutions are nonchromatographic methods, working with or without separation, such

Table 3 Comparison between capillary and packed columns for HDC

Column Type	*Length (m)*	*Diameter (mm)*	*V (mL)*	*u (cm s^{-1})*	*Shear (s^{-1})*	*ΔP (bar)*	*N*	*N (m)*	*H (cm)*	*R_F max*	*Range (mm)*
Capillary	120	0.25	5.9	34	10^{-4}	175	1500	12.5	8	1.45	0.8–20
packed	0.5	5	7.4	0.1	10^{-8}	35	7000	14 000	0.007	1.15	0.05–1

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as dynamic light scattering (photocorrelation spectroscopy) and sedimentometry under centrifugation in a disc. In the presence of SDS, at low ionic strength, HDC results in the range of 200 nm are slightly higher but close to disc centrifuge values. The latter method is of high resolution, since the separation time is inversely proportional to the diameter squared. Analysis time is low in photocorrelation spectroscopy (1 min) and is similar for HDC and centrifugation in a disc. Photocorrelation spectroscopy operated with a proper choice of parameters, gives an excellent correlation with transmission electron microscopy values and an excellent resolution for the mixture of two latexes.

Mechanism of Separation Mode

Classical chromatographic separation occurs because of the differences in partition of compounds between mobile and stationary phases. More generally, separation is the result of local differences in distribution of the sample compounds in the mobile phase. The partition coefficient K is related to the thermodynamic relationship: $\ln K = \Delta G^{\circ}/RT$, which indicates the possible effects of three factors: enthalpy (ΔH) or entropy (ΔS) changes, and temperature T (Kelvin). Practically, separation is achieved under the effect of two forces (or fields) operating in one or two phases. One phase is necessary for transport and may have a physical or chemical role in the separation.

In HDC, separation results under the effect of one hydrodynamic field which is moving one mobile phase. The nature of the mobile phase is theoretically irrelevant, but differences in results obtained with different solvents have been observed. The separation is due to the existence of a flow–velocity profile in the channel, in which small particles tend to be closer to the external wall, where the flow is stagnant. If a packing is present, its only role is to decrease the capillary size. Voids between beads (diameter Φ_p) in packed columns play the role of small channels of continuously variable diameter similar to a set of capillaries. Bird proposed the equation:

$$R = \Phi_p \varepsilon / 3(1 - \varepsilon) \qquad [15]$$

where ε is the ratio of interstitial volume to total column volume, i.e. about 0.35–0.40, so that R is around $\Phi_p/5$–6. In consequence, the mechanism of HDC in packed or capillary columns may be described by the same parallel capillary model.

The velocity profile $u(r)$ is parabolic, obeying Poiseuille's equation:

$$u(r) = \mathrm{d}P(R^2 - r^2)/4\mu\,\mathrm{d}L \qquad [16]$$

where $\mathrm{d}P$ and $\mathrm{d}L$ are increments of pressure and column length, R is the column radius, r is the particle radius and μ is the eluent viscosity. It is easy to see that $u(0)$ is a maximum when $u(R)$ is zero. The average fluid velocity, $\bar{u}$, is:

$$\bar{u} = \frac{\int_0^R u(r) r\,\mathrm{d}r}{\int_0^R r\,\mathrm{d}r} = \frac{R^2\,\mathrm{d}P}{8\mu\,\mathrm{d}L} \qquad [17]$$

which is half that of the maximum. A particle in the fluid is assumed to have the same velocity as the flow, in its gravity centre, and moving from the column to a distance $R - r$ of the wall:

$$\bar{u}_p = \frac{\int_0^{R-r} u(r) r\,\mathrm{d}r}{\int_0^{R-r} r\,\mathrm{d}r} = \bar{u}\left\{1 + \frac{2r_p}{R} - \left(\frac{r_p}{R}\right)^2\right\} \qquad [18]$$

Taking into account the definition of R_F, we derive:

$$R_F = \frac{\bar{u}_p}{\bar{u}} = 1 + 2\left(\frac{r_p}{R}\right) - \left(\frac{r_p}{R}\right)^2 \qquad [19]$$

An additional term expresses the rotational motion, so that the velocity profile is:

$$\frac{\bar{u}_p}{\bar{u}} = 1 + 2\left(\frac{r_p}{R}\right) - \gamma\left(\frac{r_p}{R}\right)^2 \qquad [20]$$

where γ is a wall effect parameter, the value of which depends upon the radial position of the particle (from about 1 to 60). In capillary HDC R_F must be independent of length L, but the coefficients of the equation are quite far from the theoretical values. This expresses the fact that R_F is higher than expected and that particles move far from the wall.

For small r_p/R (about 0.1), R_F is a linear function of r_p/R. In fact, the corresponding curve tends rapidly to a plateau value. This means that this equation is valid for one particle and is only the result of the hydrodynamic effect. If allowance is made for non-zero stagnant volume, another term is required to complete the above equation. It includes the fraction K of stagnant volume available for polymer and V_S and V_m, stagnant and mobile volumes, respectively:

$$u_p = \Delta P\{(R^2 - (R - r_p)^2 - \gamma(r_p^2)\}/\{8\mu L(1 + KV_S/V_m)\} \qquad [21]$$

The concentration profile of particles, $C_p(r)$, resulting from Brownian diffusion and colloidal interactions, must also be taken into account:

$$\overline{u_p} = \frac{\int_0^{R-r} u_p(r) C_p(r) 2\pi r\,\mathrm{d}r}{\int_0^{R-r} C_p(r) 2\pi r\,\mathrm{d}r} \qquad [22]$$

where:

$$C_p(r) = A \exp(-\phi(r)/K_B T) \qquad [23]$$

after a sufficient diffusion in the column. K_B is the Boltzmann constant and $\phi(r)$ an energy term depending on packing and particle interactions. ϕ is the resulting sum of repulsive double-layer and attractive van der Waals forces (Born repulsive forces are of negligible effect). A graphical representation of the overall profile of particle concentration is shown in Figure 2B. Van der Waals forces depend on the Hamaker constant and double-layer forces depend on latex surface potential and dielectric constant of particles and packing. The result is:

$$\overline{u_p} = \frac{\int_0^{R-r} u_p(r) \exp\left(-\dfrac{\phi(r)}{K_B T}\right) r \, dr}{\int_0^{R-r} \exp\left(-\dfrac{\phi(r)}{K_B T}\right) r \, dr} \qquad [24]$$

The exponential term may be corrected by another one accounting for the particle migration under inertial hydrodynamic force and electrokinetic lift effects. Many experiments have been carried out to fit elution (volume and peak width) results and equations, by adjusting these values. Listing of the results is more relevant to colloid chemistry than HDC.

Another effect must be considered, described as early as 1836 when Poiseuille observed a corpuscle-free region near the wall in blood vessels. More precisely, Taylor observed an uneven distribution of erythrocytes in flowing blood: there was low concentration not only near to the wall but also near to the centre, provided the velocity was large enough. Further studies concluded that radial forces tend to carry a rigid sphere to an equilibrium position at approximatively $0.6R$, depending on the velocity and on the ratio r/R. This was called the tubular pinch effect. Experiments were done with large spheres (0.16–0.85 mm radius in a tube of radius 5.6 mm, at a velocity from 5 to 90 cm s^{-1} and viscosity from 17 to 410 cP. Ploehn assumed that lateral migration of small particles is primarily due to diffusion, while large particles are focused by the inertial force at an equilibrium position, as observed in the tubular pinch experiment. This experiment may be responsible for separation in the capillary column (see above).

Resolution

We now turn to comparison of resolution (R_S) in the different fractionation processes: we shall examine R_S value and its variation with elution volume.

Table 4 Resolution of various fractionation methods

Parameter	*Capillary HDC*	*HDC*	*DCP*	*TFFF*	*SFFF*	*SFC*
R_S	0.15	0.15	1.5	0.7–2	2.2	1.2
R_t	0.025	0.025	0.15	0.2–0.02	0.07	0.03
t (min)	6	6	10	45–120	30	20–60
ϕ (μm)	>1	<1	<60	<1	<2	0.05

Reprinted with permission from Revillon A (1994) *Journal of Liquid Chromatography* 17: 2991–3023. CHDC, Capillary HDC; DCP, centrifugation under disc; TFFF, Thermal FFF; SFFF, steric FFF; SFC, supercritical fluid chromatography.

Firstly, we define a specific resolution $(R_{sp}) = R_s$/ratio of the diameter of the species under consideration. Secondly, we define resolution per unit time $(R_t) = R_{sp}$/time of measurement, in order to compare different samples and different elution conditions, respectively. To obtain polymer dimensions in solution, molecular mass, M, is converted to diameter, applying the equation: diameter is proportional to $M^{0.6}$.

R_S depends on chemical or physical factors, packing size being one of them. As a general rule in chromatography, resolution increases when the particle size of the packing decreases. In HDC with packed columns, the R_F ratio is increased by using fine packings and resolution is increased. Pore size and pore volume, being the origin of molecular separation in SEC, strongly affect elution, whereas the effect of porosity is controversial in HDC, where the main phenomenon takes place essentially between – and not in the porous part of – the packing particles. Combined HDC and SEC action increases separation, in terms of sample mass and elution volume (see above).

For SEC, HDC, supercritical fluid chromatography and TFFF, theoretical plate height generally increases with flow rate according to the van Demter equation, so that resolution decreases. Sometimes R_t may increase, particularly if a gradient is used properly or when mass transfer does not play a role.

For all separations, it is possible to obtain an accurate molecular weight by using deconvolution to remove system dispersion.

Table 4 indicates meaningful values of R, R_S, R_t and analysis time with the various separation systems.

Conclusion

HDC is a complementary method for particle characterization in the micron size range. It is rapid, low cost, easy to carry out and very sensitive. It allows separation and size measurement of organic colloids,

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soft and rigid polymers and various materials (spheric or elongated) in about 10 min, without special sample preparation. Moreover, it has been the subject of many theoretical studies, assuming different mechanism models and explaining the effect of parameters on separation. Effort has been directed to column design, detection sensitivity and fit of chromatogram for PSD. Some conditions must be obeyed to obtain reliable results, whatever columns are used. Firstly, there is the need for accurate calibration, with frequent adjustment of parameters and choice of a column set giving a low slope. Secondly, there is the need to adjust the detector response factor (exponent n of diameter) to the sample and to the chosen detector. Thirdly, axial dispersion must be corrected using a simple equation. As a consequence of the combined analysis of the effects of n and Pe, the proposed value for exponent n was 3.5 with UV detection and a Peclet number of 500. Results have been compared on eluted fractions with those of direct methods. With proper use of HDC interpretation parameters, PSD and average diameters are in agreement with those obtained by other methods, for instance photon correlation spectroscopy and sedimentometry, this last technique has high resolution as its main characteristic.

In packed columns, packing size, ionic strength, I, nature and amount of surfactant have very large effects on R_F and other elution parameters, such as percentage recovery, which decreases for large particles. R_F decreases when I or packing size or amount of surfactant increases. Porous packings may add a SEC separation effect, enhancing R_F. Improvements in packing material (5 μm) and methodology tend to use a 0.5 m long column.

In capillary HDC, the effect of additives is low; surfactants are the most useful. The separation factor R_F is fairly constant in water but is strongly affected by flow rate in methanol. The high number of plates in tetrahydrofuran is not accompanied by a high maximum R_F value. This separation factor may be higher with a 60 m than with a 120 m column, but the resolution, R_S, is not so high. This emphasizes the fact that R_F, R_S and N may vary independently.

Micro-equipment (diameter of the capillary in the micron range, short column (10 m), on-column injection and detection) enhances resolution and R_F as it lowers axial dispersion. Peak capacity remains limited at about 10, but for rheological properties and size range, this is an interesting alternative to packed columns.

See also: **I/Particle Size Separation. II/Chromatography: Liquid:** Mechanisms: Size Exclusion Chromatography. **Particle Size Separation:** Electrostatic Precipitation; Field Flow Fractionation: Thermal. **III/Polymers:** Field Flow Fractionation. **Proteins:** Field Flow Fractionation.

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